REMARKS

Interview request

Applicants respectfully request a telephonic interview after the Examiner has reviewed the instant response and amendment. Applicants request the Examiner call Applicants' representative at (858) 720-5133.

Status of the Claims

Pending claims

Claims 31, 34, 35, 114, 115, 132 to 154 and 189 to 201 are pending.

Outstanding Rejections

The rejection of claims 31, 34, 114, 115, 134, 140-154 and 189-200 is maintained and claim 201 is newly rejected under 35 U.S.C. §112, second paragraph. The rejection of claims 31, 34, 114, 115, 134, 140-154 and 189-200 is maintained and claim 201 is newly rejected under 35 U.S.C. §112, first paragraph, enablement requirement. The rejection of claim 193 under 35 U.S.C. §112, first paragraph, written description requirement, is maintained. Applicants respectfully traverse all outstanding objections to the specification and rejection of the claims.

The Applicants respectfully note that all pending claims are free of the cited art.

Support for the Claim Amendments

The specification sets forth an extensive description of the invention in the new and amended claims. Support for claims directed to methods for making a polypeptide polymer made by self-assembly of monomers, wherein at least one monomeric polypeptide of the plurality of monomeric polypeptides has a modification comprising attachment of an enzyme, attachment of a nucleotide or attachment of a nucleotide derivative or attachment of a lipid or attachment of a lipid derivative or attachment of a targeting vector, can be found, inter alia, in paragraph spanning pages 7-8. Support for claims directed to methods for making a polypeptide polymer made by self-assembly of monomers, wherein the polypeptide polymer is a nanoscale drug delivery vehicle can be found, inter alia, on page 3, lines 17 to 18; page 89, lines 17 to 20; page 90, lines 17 to 20.

Sequence Listing

As requested a new sequence listing has been submitted (sent under a separate cover to "mail stop sequence" March 23, 2005).

Information Disclosure Statements

Applicants thank the Examiner for considering and initialing the patents listed on the supplemental Information Disclosure Statement (IDS) submitted herein and on the IDSs submitted February 21, 2003 and May 29, 2002. It is respectfully requested that the cited information be expressly considered during the prosecution of this application, and the references be made of record therein and appear among the "references cited" on any patent to issue therefrom.

Issues under 35 U.S.C. §112, second paragraph

The rejection of claims 31, 34, 114, 115, 134, 140-154 and 189-200 is maintained and claim 201 is newly rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter that the Applicants regard as the invention.

Regarding claim 34, on pages 2 to 3, paragraph 3B (see the instant office action, mailed September 09, 2004 ("OA"), the Office alleged that the claim is indefinite because it is not clear how the method can produce polypeptides with attachments. The instant amendment addresses this issue.

Regarding claim 150, page 3, paragraph 3D, the Office alleged that the claim is indefinite because it is not clear how attaching of a targeting molecule can be recited as a further step if the base claim requires that such targeting molecule has already been attached. The instant amendment addresses this issue. Please note that attaching of a targeting molecule is only one of several options in the base claim – an enzyme, a nucleotide or nucleotide derivative, a lipid or lipid derivative or a vector can also be attached. Thus, in one aspect, if an enzyme, a nucleotide or nucleotide derivative, a lipid or lipid derivative and/or a vector has been attached, then it is not redundant to "further comprise" attaching a targeting moiety. However, if a particular kind of targeting molecule has already been attached, a second targeting moiety can be also be attached, as

set forth in claim 150. The instant amendment clarifies this point. The same explanation applies to vectors added to the therapeutic agent- or drug-loaded polymer.

The Office also alleges that it is further unclear where and how the targeting molecule is being attached. However, the specification expressly teaches alternative exemplary ways to attach a targeting molecule, or an enzyme, a nucleotide or nucleotide derivative, a lipid or lipid derivative and/or a vector, e.g., on page 90, line 17 to page 91, line 3:

> In order to better direct the nanoscale drug delivery vehicle or polymer of the present invention to a particular desired location in an animal body, a targeting vector may be attached to the polymer or the monomeric polypeptide of the present invention. The targeting vector useful in the present invention includes antibodies, oligosaccharides, and Morphatides™. All of these targeting vectors may be readily attached to the monomeric polypeptide surface using conventional chemistries. Antibodies are the most common targeting vectors but oligosaccharides have also been shown to function as effective targeting moieties (see Wu, *Evidence for targeted gene delivery to HepG2 hepatoma cells in vitro,* V: 27, no. 3, pp. 887-892 (1988); Hashida, Akamatsu, Nishikawa, Fumiyoshi, Takakura, *Design of polymeric prodrugs of prostaglandin E_1 having galactose residue for hepatocyte targeting*, J. Controlled Release: v. 62, pp. 253-262 (1999)). The presence of a plurality of potential N-linked glycosylation sites in the monomeric polypeptide makes glycosylation-based targeting an attractive approach. In addition, Morphatides™ may be attached to the monomeric polypeptide using common synthetic methods. Morphatides™ is a derivatized nucleotide complex that may be optimized through iterative *in vitro* evolution to bind specific antigens. (emphasis added)

The specification expressly teaches another alternative means to attach a targeting molecule to a monomeric polypeptide used in the methods of the invention, e.g., on page 98, line 27, to page 99, line 8 (e.g., as a fusion protein):

> A polynucleotide sequence selected from SEQ ID NOS. 1, 3, 5, 7, and 9 and sequences substantially identical or complementary thereto, and fragments thereof may be further modified by incorporating one or more sequences encoding one or more antigens therein using a suitable gene modification method such as recombinant DNA or a method described above. In this method, the one or more sequences encoding one or more antigens are inserted into the polynucleotide sequence so that when the polynucleotide sequence is expressed to produce a polypeptide, the antigen or antigenic

> domain is exposed on the surface of the expressed polypeptide. In a more preferred embodiment, when expressed polypeptide is assembled or self-assembled into a polymer of the present invention, the antigen or antigenic domain is exposed on the surface of the polymer. (emphasis added)

The specification expressly teaches how to attach a lipid molecule to a polypeptide used in the methods of the invention, e.g., on page 101, lines 12 to 24 (note the attaching of the lipid is non-covalent):

> In another embodiment of encapsulating one or more drugs, in addition to the monomeric polypeptide units, lipids or lipid molecules are used to encapsulate a drug molecule. In this embodiment, liposomes are induced to form from lipids in the presence of both the drug molecules and the monomeric polypeptide units, preferably in a solution, in the presence of a divalent cation such as millimolar calcium and magnesium as described in Akasji et al, *Formation of giant liposomes promoted by divalent cations: critical role of electrostatic repulsion*, Biophys. J. v. 74, pp. 2973-2982. The formed liposomes encapsulate one or more drug molecules and monomeric polypeptide units therein. After the formation of the liposomes, the condition of the mixture or solution containing the liposomes is changed to, for example, a higher temperature to induce the assembly of the monomeric polypeptide units into polymers or nanotubes to produce a complex wherein the one or more drug molecules are encapsulated in the polymer or nanotube with a lipid coating. (emphasis added)

The specification expressly teaches how to attach small molecules to a polypeptide used in the methods of the invention, e.g., on page 104, lines 15 to 28:

> In a preferred embodiment, the biochip of the present invention uses a hydrogel based on a self-assembling polymer in accordance with the present invention. Alternatively, a the hydrogel may be based on a prepolymer of polyethyleneoxide, or a copolymer of polyethyleneoxide and polypropyleneoxide, capped with water-active diisocyanates and lightly cross-linked with polyols such that the quantity of isocyanates present is predictable for example is at most about 0.8 meq/g. Frequently used diisocyanates include aromatic-based diisocyanates, such as toluene diisocyanate or methylene diphenyl-isocyanate, as well as aliphatic diisocyanates, such as isophorone diisocyanate. The polymerization of the prepolymer, which may be preformulated in water-miscible organic solvent, takes place simply by the addition of water. One advantage of the water-activated polymerization and/or the self-assembly polymerization methods of the present invention is that they

> allow for derivatization of the pre-polymer with an appropriate biomolecular probe prior to or simultaneously with polymerization. (emphasis added)

The specification provides another exemplary means to attach small molecules (an unsaturated side chain such as a styrene moiety or a divinylbenzene) to a polypeptide used in the methods of the invention, e.g., on page 109, lines 3 to 14:

> In one preferred embodiment of the method of using the polymer as a separation agent according to the present invention, the polymer may modified by introducing an unsaturated side chain such as a styrene moiety using common synthetic methods such glycosylation using a styrene substituted glycoside. Thereafter, the modified polymer may be copolymerized with styrene and divinylbenzene using emulsion or suspension polymerization methods to form a universal chiral separation resin with the polymer covalently attached to the resin. Alternatively, the styrene and divinylbenzene may be copolymerized in the presence of an unmodified polymer of the present invention to form a resin with the polymer being non-covalently attached. The resin is then packed into an HPLC column and the packed column is installed in a HPLC system to be used to separate pharmaceutical compounds. (emphasis added)

Accordingly, the specification clearly teaches where and how a targeting molecule, or an enzyme, a nucleotide or nucleotide derivative, a lipid or lipid derivative and/or a vector, can be attached to a polypeptide used in the methods of the invention.

Regarding claim 193, Applicants thank the Examiner for clarifying his concerns. The instant amendment addresses this issue.

Issues under 35 U.S.C. §112, first paragraph

Enablement

The rejection of claims 31, 34, 114, 115, 134, 140-154 and 189-200 is maintained and claim 201 is newly rejected under 35 U.S.C. §112, first paragraph, as allegedly not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention.

The Office maintained this rejection based on reasons of record and further in view of argument set forth in the OA.

While Applicants aver that the specification does provide reasonable enablement to the skilled artisan, they first respectfully submit that the Patent Office has not met its initial burden to establish a reasonable basis to question the enablement provided for the claimed invention, and specifically address below how the art used to support the Office's enablement rejection is not sufficient to rebut the presumptively enabled specification.

In order to make a rejection, the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. In re Wright, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (Examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure). A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. As stated by the court, "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). See also MPEP §2164.04, 8th ed., rev. 2, May 2004, pg 2100-189.

To support its *prima facie* case of lack of enablement, the Office alleged (see office action mailed May 15, 2004, page 6, lines 14 to 18), inter alia, that "[i]t is well known that the process of self-assembly of polypeptide monomers into polymers depends critically on the structure of the monomers and even slight changes, such as change in length of chain, or addition of ionized residue may change the rate and/or direction of the reaction." Two articles were cited in support of

this allegation: Urry, et al. (1992) Materials Res. Society Symposium Proceeding 255:411-422 ("Urry"); and, the abstract Jenekhe, et al. (2000) ACS, 220th, PMSE-268 (Abst. 268) ("Jenekhe") (see Form 892, prepared with the office action mailed May 15, 2004). However, neither of the references cited by the Office establish a reasonable basis to question the enablement provided for the claimed invention, for reasons set forth below, and, as submitted by Applicants in their response of August 27, 2004, expressly incorporated herein.

The Urry article, entitled "Hierarchial and Modulable Hydrophobic Folding and Self-Assembly in Elastic Protein-Based Polymers: Implications for Signal Transduction," discusses folding and self-assembly properties of hydrophobic (apolar) and polar moieties of elastomeric polypeptides, in particular, elastic protein-based polymers, such as elastin. The effects of changes in amino acid sequence, ionization conditions (e.g., pH) and temperature on the hierarchical hydrophobic folding of a series of elastomeric polypentapeptides was studied. Urry concluded that hierarchical hydrophobic folding can be modulated by changing the degree of ionization or by changes in a number of intensive variables. Urry noted that changes in these variables can be used to drive folding/unfolding-assembly/disassembly transitions under isothermal conditions, and that these folding/unfolding-assembly/disassembly transitions can be used to achieve signal transduction (i.e., the translocation or transduction of free energy). Urry also concluded that the process of folding and the formation of tertiary and quaternary structures could be readily and predictably controlled by manipulating temperature and the degree of hydrophobicity in the polypentapeptide, see, e.g., the first paragraph, page 413, of Urry. It appears Urry was able to easily and simply identify changes in protein sequence and folding conditions to drive a protein polymer assembly or disassembly reaction. In fact, Urry's data and conclusions actually support the idea that folding/unfolding-assembly/disassembly of a protein can be easily manipulated by readily identified and manipulated variables. Thus, Urry is not a reference that can establish a reasonable basis to question the enablement provided for the claimed invention.

The Jenekhe abstract, entitled "Self-assembly of Functional Mesostructures and Discrete Objects from Synthetic Polymers," summarizes their work regarding the formation of functional mesostructures. They noted that they synthesized amphiphilic rod-coil block co-polymers of

diverse macromolecular architecture. Jenekhe noted that they found these polymers to self-organize into ordered supramolecular assemblies, e.g., vesicles, microtubules, smectic layers and periodic microporous nanostructured thin films. They noted that novel cooperative properties and tunable optoelectronic and photonic properties were observed in their self-organized, supramolecular assemblies. Jenekhe concluded that their results demonstrated the potential of self-assembling polymers for engineering complex, functional and three-dimensional periodic mesostructures of discrete objects. Jenekhe did not note any problems or technological difficulties in making their self-organized, supramolecular assemblies. Jenekhe's data and conclusions actually support the idea that self-assembling polymers can be used for engineering complex, functional and three-dimensional periodic mesostructures into discrete objects. Thus, Jenekhe is not a reference that can establish a reasonable basis to question the enablement provided for the claimed invention.

Applicants respectfully maintain that the Office has not met its initial burden to establish a reasonable basis to question the enablement provided for in the specification for the claimed invention. Accordingly, the Patent Office has not set forth a *prima facie* case of lack of enablement and the rejection under section 112, first paragraph should be withdrawn.

The Office has maintained the section 112, first paragraph, enablement rejection, and has provided further reasons of record, including addressing the Rule 132 declaration of Dr. Barton, see, e.g., pages 4 to 7, paragraph 4, of the OA. Applicants respectfully maintain that the specification does provide reasonable enablement to the skilled artisan to make and use the claimed invention.

Regarding the further reasons of record, the Office is concerned about the sufficiency of information specifying what kind of "protein polymer" is exemplified in Figure 1 (see, e.g., third paragraph section 4., page 4, of the OA). To address these concerns Applicants submit a second Rule 132 expert declaration by co-inventor Dr. Nelson Barton (whose credentials were submitted with his first declaration in Applicants' last response of August 27, 2004), who declares that Figure 1 in this application is an illustration of a transmission electron micrograph of a self-assembled protein polymer of the invention, where the protein as a monomer has a sequence as set forth in

SEQ ID NO:2. Accordingly, the Office can accept the assertion that the polymer illustrated in Figure 1 is the self-assembled protein having a sequence as set forth in SEQ ID NO:2.

The Office alleges that Example 19 of the specification, referenced in Dr. Barton's Rule 132 declaration submitted with Applicants' last response, is unclear as to the meaning of the term "CanA". In support, a portion of a sentence from the specification is cited (see page 5, lines 1 to 9, of the OA). Applicants respectfully submit in reading the paragraph from which the cited sentence fragment was taken in its entirety, in context with the specification as a whole, the skilled artisan would understand that CanA can mean either the exemplary polypeptide having a sequence as set forth in SEQ ID NO:2, or, an exemplary nucleic acid (SEQ ID NO:1) that encodes it depending, of course, on the context in which the term is used:

> Preferably, the nucleic acid has a sequence as set forth in the Group A nucleic acid sequences or may be produced by modifying a nucleic acid having a sequence as set forth in the Group A nucleic acid sequences and sequences substantially identical thereto using the methods described below. Group A nucleic acid sequences and the Group B amino acid sequences, which are encoded by Group A nucleic acid sequences have substantial homology. The alignment for the corresponding Group A nucleic acid sequences and Group B amino acid sequences using a common bioinformatic algorithm or an algorithm discussed above is shown below. In the following alignment, CanA and CanA_pep stand for nucleic acid SEQ ID No. 1 and its corresponding amino acid SEQ ID No.2, respectively; CanB and CanB_pep stand for nucleic acid SEQ ID No. 3 and its corresponding amino acid SEQ ID No. 4, respectively; CanC and CanC_pep stand for nucleic acid SEQ ID No. 5 and its corresponding amino acid SEQ ID No. 6, respectively; CanD_partial stands for nucleic acid SEQ ID No. 7 or its corresponding amino acid SEQ ID No. 8; and CanE partial stands for nucleic acid SEQ ID No. 9 or its corresponding amino acid SEQ ID No. 10. (emphasis added) (from page 15, line 26, to page 27, line 11).

Thus, the sentence fragment cited by the Office is in fact referring to nucleic acid and polypeptide amino acid alignments set forth in the specification on page 16, line 12 to page 18, line 35. The specification expressly clarifies that SEQ ID NO:2 is an amino acid sequence, see, e.g., page 17, last line ("Amino Acid Alignment for SEQ ID NOS. 2, 4, 6, 8, and 10:"). See also Example 20, page 151, lines 14 to 17, of the specification:

> The polymer may have a shape of a short fiber, and therefore is also called "polymer fiber." The polymer fiber is made from monomeric protein units (e.g. Can A: 182 amino acids: MW = 19,830 Daltons, having a sequence of SED ID NO. 2). The secondary structure of the protein may be mainly β-sheets. (emphasis added)

Additionally, in his Rule 132 declaration submitted with Applicants' last response, Dr. Barton declared that Examples 19 and 20 of the specification set forth experimentation demonstrating that SEQ ID NO:2 monomers can self-assemble into polymers. Dr. Barton declared that these examples, together with the associated disclosure throughout the specification, would have been sufficient to demonstrate how to make and use the presently claimed invention to one of skill in the art.

To further address this issue, in the declaration submitted with this response, Dr. Barton declares that at the time of the invention the skilled artisan, after reading the specification in its entirety, would have understand that CanA can mean either the exemplary polypeptide having a sequence as set forth in SEQ ID NO:2, or, an exemplary nucleic acid (SEQ ID NO:1) that encodes it - depending, of course, on the context in which the term is used. Dr. Barton also declares that Examples 19 and 20 of the specification set forth experimentation demonstrating that SEQ ID NO:2, also called CanA, can self-assemble into polymers. Dr. Barton declares that Examples 19 and 20, together with the specification, would have been sufficient to demonstrate to one of skill in the art how to make and use the invention including the polymerization of CanA, or SEQ ID NO:2.

Regarding Example 20 (pages 149 to 151 of the specification), which – as declared by Dr. Barton in his previous declaration - sets forth experimentation demonstrating that SEQ ID NO:2, can self-assemble into polymers, the Office alleges there is no disclosure that the polymer is comprised of purified SEQ ID NO:2 because there is no disclosure that purification went as far as dividing the extract into fractions containing individual proteins (see, inter alia, page 5, second paragraph, of the OA). This issue is addressed by Dr. Barton in his previous declaration, where he declares that it would be evident to one of skill in the art that Example 20 contains two precipitation/centrifugation steps that purify the extract before polymerization. *See* pages 149-150 of the specification. Dr. Barton declared that the first precipitation is performed utilizing heat treatment, which denatures and precipitates the *E. coli* proteins [see, e.g., page 149, lines 24 to 30]. Dr. Barton

declared that these proteins are then removed by centrifugation, which leaves a soluble CanA fraction that self-assembles [see, e.g., page 150, lines 1 to 3]. Dr. Barton declared that as CanA comes from a hyperthermophile, it can withstand the heat treatment step. Dr. Barton declared that Example 20 describes the performance of additional ammonium sulfate precipitation, followed by another centrifugation [see, e.g., page 150, lines 6 to 14], and that this precipitation/centrifugation step further purifies the supernatant/extract. Accordingly, Dr. Barton declared that Example 20 describes an appreciably different process than the polymerization of a crude *E. coli* extract.

However, in its comments regarding Example 20, the Office (see page 5, second paragraph, of the OA) completely disregards co-inventor Dr. Barton's declaration. The examiner must weigh all the evidence before him or her, including the specification and any new evidence supplied by applicant with the evidence and/or sound scientific reasoning previously presented in the rejection and decide whether the claimed invention is enabled. The examiner should never make the determination based on personal opinion. The determination should always be based on the weight of all the evidence. MPEP §2164.05, 8th edition, rev. 2, May 2004, pg 2100-190 to -191. Because the examiner must weigh all the evidence before him or her, the Office did not sufficiently consider and address Dr. Barton's previously submitted Rule 132 expert declaration regarding enablement, as discussed above, with reasons to doubt the objective truth of the statements contained therein. Applicants respectfully aver that Dr. Barton's expert declaration with the specification is a sufficient showing that Example 20 sets forth a disclosure demonstrating that a method to purify SEQ ID NO:2, or CanA, and the polymer made in the example from the purified monomer is comprised of polymerized SEQ ID NO:2, or CanA. The evidence provided by applicant need not be conclusive but merely convincing to one skilled in the art. MPEP 2164.05, 8th edition, rev. 2, May 2004, pg 2100-190 to -191.

The specification also summarizes the CanA purification procedure set forth in Example 20, inter alia, on page 22, line 9 to 17, describing it as a process for preparing monomeric polypeptides of the invention:

> ... the process of preparing monomeric polypeptides or polypeptide units of the present invention further includes a fourth step of isolating the

> produced polypeptide from the culture or medium. The step of isolating the monomeric polypeptide can be carried out by French pressing the *E. coli* cell mass from a solution, removing particles from the solution by centrifugation, heat-treating the solution to precipitate the unwanted heat-sensitive proteins, centrifugating the heat-treated solution to obtain a clear solution, precipitating the monomeric polypeptides from the clear solution using ammonium sulfate and dialyzing the monomeric polypeptides to reduce the ionic strength of the solution.

The Office also alleges that there is no mention of any particular protein in Example 20 (see page 5, last sentence, second paragraph, of the OA). However, please see page 149, lines 1 to 10, of Example 20:

> Production Of The Polymer Of The Present Invention.
>
> a) 300L Fermentor Culture of Recombinant E. Coli.
>
> A 300 L culture of recombinant E. coli BL21 (DE3) harboring expression plasmid pEX-CAN-A (produced by attaching sequence substantially identical to SEQ ID NO. 1 to a vector pET17b using a procedure described in Example 20) was grown in a HTE-Fermentor (Bioengineering, Wald, Switzerland) at 37°C under aeration (165 L air / min.) and stirring (400 rpm) with a doubling time of about 40 min. At an O.D. (600nm) of 0.80, production of Can A protein was induced by addition of 30 grams of IPTG. Cells were harvested 3 hours after the induction and after being cooled down to 4°C. Cell yield: 1,610 grams (wet weight). (emphasis added)

Thus, Example 20 expressly references use of CanA, encoded here by SEQ ID NO:1, which has the sequence as set forth in SEQ ID NO:2, as noted elsewhere in the specification – see discussion above. See also Example 20, page 151, lines 14 to 17, of the specification:

> The polymer may have a shape of a short fiber, and therefore is also called "polymer fiber." The polymer fiber is made from monomeric protein units (e.g. Can A: 182 amino acids: MW = 19,830 Daltons, having a sequence of SED ID NO. 2). The secondary structure of the protein may be mainly β-sheets. (emphasis added)

The Office (see, page 5, last paragraph, of the OA) also alleges that Example 20, section vi. (page 150, line 16 to page 151, line 4), describes polymerization in the presence of unidentified "polymer primers," prepared from a polymer suspension, and thus allegedly does not seem to be an example of self-assembly. However, Example 20 (page 150, line 16 to page 151, line 4) expressly and clearly describes "polymer primers":

> The dialyzed protein solution was diluted by addition of buffer to a final protein concentration of 6.5 mg/ml (final volume 325 ml). Then, under shaking in a 1L Erlenmeyer flask at 100°C (in a water bath), the diluted protein solution was rapidly heated to 80°C and then immediately transferred into a 500 ml screw-capped storage bottle. The storage bottle contained 3.32 ml (21.58 mg protein) of "Polymer Primers" (the "Polymer Primers" had been prepared before by 4 times French Press-shearing of a prefabricated Polymer suspension). Then, CaCl and MgCl (each at 20 mM final concentration) were added to the mixture and the closed bottle was stored in an 60°C water bath. After addition of these salts, the solution became immediately turbid, indicating rapid polymerization of the protein units. After 10 min polymerization, the formed Polymer fibers were sheared by ultraturraxing the solution for 20 seconds in order to create additional polymer primers to speed up polymerization. Traces of silicone antifoam may be added before the ultraturraxing to reduce foaming. Typically, after 10 min. polymerization at 80°C, Polymer or polymer fibers could be observed under an electron microscope. After 1 to 2 hours of polymerization, protein polymers could be completely removed from the solution by centrifugation (15 min., 20,000 rpm, Sorvall rotor SS34), indicating complete polymerization. (emphasis added)

Thus, "Polymer Primers" (or "templates", see below") are made by French Press-shearing of a prefabricated polymer of the invention. This exemplary method is further discussed, inter alia, on page 99, lines 12 to 27:

> In another aspect, the present invention provides a method of producing a polymer including a plurality of the monomeric polypeptide units of the present invention. In the method of producing the polymer of the present invention, a plurality of the monomeric polypeptide units are polymerized under suitable conditions to form the polymer. Preferably, the monomeric polypeptide units are polymerized in the presence of a template molecule. More preferably, the monomeric polypeptide units are polymerized through a self-assembly process in the presence of at least one divalent cation. In a preferred embodiment, the at least one divalent cation may be selected from the group consisting of Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Sr^{2+}, Ni^{2+}, Mn^{2+} and Fe^{2+}. In a more preferred embodiment, the at least one divalent cation includes Ca^{2+}. In a most preferred embodiment, the at least one divalent cation includes both Ca^{2+} and Mg^{2+}. Most preferably, the method of producing the polymer involves: dissolving the monomeric polypeptides in an aqueous solution, adding the aqueous solution containing the monomeric polypeptides to a container having at least one template molecule and adding Ca^{2+} and Mg^{2+} solutions to the container to polymerize the monomeric polypeptides to form the polymer.

> The template molecule used in the present invention may be selected based on the desired properties of the polymer. In a preferred embodiment, the template molecule is prepared by French Press-shearing of a suspension of the polymer of the present invention. (emphasis added)

The Office also alleges that there is no evidence that the product of the method described in Example 20 is a polymer is a peptide (see page 6, lines 1 to 4, of the OA). However, this issue was fully addressed by Dr. Barton's declaration in Applicants' last response, where he declared that Examples 19 and 20 of the specification set forth experimentation demonstrating that SEQ ID NO:2 monomers can self-assemble into polymers, and that these examples, together with the associated disclosure throughout the specification, would have been sufficient to demonstrate how to make and use the presently claimed invention to one of skill in the art. Dr. Barton also declared that these polymer fibers are comprised of the polypeptide of SEQ ID NO:2.

With regard to Example 21, the Office alleges that the example does not describe polypeptide polymer comprising tubules, rather it describes lipid tubules containing drug molecules, citing page 152, line 14, of the specification. It is also alleged that there is no indication that any polymerization of SEQ ID NO:2 is occurring (see page 6, lines 5 to 18, of the OA). However, if read in its entirety (page 152, lines 1 to 14), Example 21 clearly describes a polypeptide polymer acting as a drug delivery complex where the lipid is a coat to a drug delivery complex comprising a polymerized SEQ ID NO:2:

> Preparation of Lipid Coated Drug Delivery Complexes
>
> To a solution containing 3 mg/ml monomeric protein units (e.g. Can A: 182 amino acids: MW = 19,830 Daltons, having a sequence of SED ID NO. 2), a desired amount of drug molecules, and a sufficient amount of electrically neutral lipids, millimolar calcium and magnesium cations are added to form a mixture. The mixture is kept at ambient condition for a sufficient amount time until liposomes form. Thereafter, gel filtration chromatography is carried out on the mixture and the liposomes contained in the mixture are size fractionated. The desired fractions of the liposomes are then heated to 50°C in the presence of millimolar amounts of calcium and magnesium cations to initiate the polymerization of the monomeric polypeptide units within each liposome. The polymerization results in the extreme deformation of the liposomes and produces sealed lipid tubules containing the drug molecules. (emphasis added)

The method of Example 21, entitled "Preparation of Lipid Coated Drug Delivery Complexes," clearly describes polymerization of monomeric polypeptide units within liposomes. As Dr. Barton declares, polymerization of CanA monomer is initiated after formation of the liposome (see, e.g., page 152, lines 10 to 14, of the specification). In fact, polymerization results in the extreme deformation of the liposomes, as illustrated in Figure 3B and 3C. As Dr. Barton declares, Figures 3B and 3C illustrate this "liposome deformation" effect, where the liposome after polymerization of the polypeptide is deformed into a tubule-like shape, versus its natural un-deformed spherical shape. Without polymerization the lipids would remain spherical. The drug can be within the deformed "tubular" lipid or within polymerized tubule, or both. The significance of this exemplary composition of the invention is the deformation of the lipid caused by the polymerization of monomeric protein units, e.g., SEQ ID NO:2. Thus, Figure 3 is related to Example 21 in that it clearly illustrates the process set forth in Figures 3A, 3B and 3C. Dr. Barton confirms this in the attached declaration. Also, to clarify Figure 3B, the term "Pyrotex" has been used by Applicants to describe CanA polymers, i.e., polymers of SEQ ID NO:2.

The Office notes that Exhibit A of Applicants last response did not make it to the Examiner (see the paragraph spanning pages 6 to 7 of the OA). Applicants herein resubmit Exhibit A, which as declared by Dr. Barton illustrates evidence that the methods and materials set forth in the application as filed enable one of skill in the art to practice the invention as presently claimed. Dr. Barton presents for consideration an immunofluorescent light microscope image (Exhibit A) of nanotubules assembled from a polypeptide conjugate of the present invention generated by attaching the CanA open reading frame (SEQ ID NO:1) to the open reading frame of the green fluorescent protein ZSGREEN™ (BD Biosciences Clontech, Palo Alto, Calif.). As declared by Dr. Barton, these nanotubules are comprised of monomeric polypeptide subunit conjugates of SEQ ID NO:2 attached to the green fluorescent protein. Dr. Barton notes that self polymerization of the monomeric polypeptide subunit conjugates was achieved under the conditions set forth in Example 20 (pages 149 to 151, of the specification).

Finally, the Office maintains that even if there has been a demonstration of polymers obtained by self-assembly of peptide SEQ ID NO:2, there is no sufficient enablement provided for

polymers formed from conjugates of the monomer with attachments – and emphasizes that the issue is not producing any peptide polymers, but rather on conditions for self-assembly (see page 7, lines 3 to 15, of the OA). In particular, it is alleged that Applicants have not provided sufficient evidence that the level of skill in the art for self-assembly of conjugates with other biomolecules was high enough to enable self-assembly of any peptide conjugates without undue experimentation.

Applicants respectfully maintain that the specification does provide reasonable enablement to the skilled artisan to make and use the claimed invention and that they have provided sufficient evidence that the level of skill in the art for self-assembly of conjugates with other biomolecules was high enough to enable self-assembly of any peptide conjugates without undue experimentation.

For example, in Applicants' last response Dr. Barton declared that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for producing polypeptide polymers was very high. In the declaration attached herein Dr. Barton emphasizes that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for determining conditions for self-assembly of protein conjugates of the invention was also very high. Dr. Barton declares that it would have been routine for the skilled artisan at the time of the invention to select conditions that facilitated self polymerization of the claimed conjugates of the invention, whether these composition comprised monomeric polypeptides having a modification comprising attachment of an enzyme, attachment of a nucleotide or attachment of a nucleotide derivative, or attachment of a lipid or attachment of a lipid derivative, or attachment of a targeting molecule, or attachment of a vector.

As evidence that the present disclosure is enabling, Dr. Barton provides experimental data in the form of an immunofluorescent light microscope image of nanotubules assembled from a fusion protein generated by fusing the CanA open reading frame (SEQ ID NO:1) to the open reading frame of the green fluorescent protein ZSGREEN(TM) (BD Biosciences Clontech, Palo Alto, Calif.). The nanotubules are comprised of monomeric polypeptide subunit conjugates of SEQ ID NO:2 (CanA) attached to the green fluorescent protein. As declared by Dr. Barton, self

polymerization of the monomeric polypeptide subunit conjugates (the CanA- GFP chimeric protein) was achieved under the conditions set forth in Example 20.

In his previous declaration, Dr. Barton declared that using the disclosure provided in the specification (*see e.g.*, page 101, line 7 to page 102, line 3; Examples 19-21; Figures 2-3), one of skill in the art would also understand how to make and use polymers comprised of conjugated / modified polypeptide monomers. Dr. Barton declared that one of skill in the art could determine alternative conditions to achieve self-assembly of the conjugated / modified monomers given the disclosure provided in the specification. Dr. Barton declared that it was considered routine by one skilled in the art to determine what conditions to use or modify, including what modifications or attachments are possible or preferred, to produce a protein polymer tubule given the starting materials set forth in the present application. Dr. Barton declared that methods for making and screening for polypeptide polymers comprised of at least one conjugated / modified monomer of SEQ ID NO:2 were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate a genus of polypeptide polymers without need of knowing which modifications or attachments would detrimentally affect self-polymerization.

Enablement is a legal determination of whether a patent enables one skilled in the art to make and use the claimed invention. Raytheon Co. v. Roper Corp., 724 F.2d 951, 960, 220 USPQ 592, 599 (Fed. Cir. 1983). Enablement is not precluded even if some experimentation is necessary, although the amount of experimentation needed must not be unduly extensive. Atlas Powder Co. v. E.I. Du Pont De Nemours & Co., 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984); W.L. Gore and Associates v. Garlock, Inc., 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983). Whether large numbers of compositions (e.g., enzymes, antibodies, nucleic acids, and the like) must be screened to determine if one is within the scope of the claimed invention is irrelevant to an enablement inquiry. Experimentation is not considered undue, even if extensive, if it is routine or if the specification provides reasonable guidance regarding the direction of experimentation -- time and difficulty are not determinative of undue experimentation if the experimentation is routine. See PPG Indus., Inc. v. Guardian Indus. Corp., 75 F.3d 1558, 1564, 37 USPQ2d 1618, 1623 (Fed. Cir. 1996); In re Wands, 858 F.2d at 736-40, 8 USPQ2d at 1403-7; Hybritech, Inc. v. Monoclonal

Antibodies, Inc.,802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987) (acknowledging that, because practitioners in that art are prepared to screen large numbers of negatives in order to find a sample that has the desired properties, the screening that would be necessary to make additional antibody species was not "undue experimentation."). Thus, enablement is not precluded by the necessity to screen large numbers of compositions, as long as that screening is "routine," i.e., not "undue," to use the words of the Federal Circuit.

Analogously, practitioners of the sciences for the instant invention also recognized the need to screen numbers of negatives to find conditions for self-assembly of the CanA conjugates of the invention. As declared by Dr. Barton, it would have been routine for the skilled artisan at the time of the invention to screen for and select conditions that facilitated self polymerization of the claimed conjugates of the invention, whether these composition comprised monomeric polypeptides having a modification comprising attachment of an enzyme, attachment of a nucleotide or attachment of a nucleotide derivative, or attachment of a lipid or attachment of a lipid derivative, or attachment of a targeting molecule, or attachment of a vector. All were routine protocols for the skilled artisan. Thus, the skilled artisan using Applicants' written disclosure could practice the instant claimed invention without undue experimentation.

Accordingly, because the Patent Office has not set forth a *prima facie* case of lack of enablement, or, alternatively, because Applicants have submitted sufficient argument, including Dr. Barton's expert declarations, convincing to one skilled in the art that any possible *prima facie* case of lack of enablement is rebutted, Applicants respectfully submit that the pending claims meet the enablement requirements under 35 U.S.C. §112, first paragraph. In light of the above remarks, Applicants respectfully submit that amended claims are fully enabled by and described in the specification to overcome the rejection based upon 35 U.S.C. §112, first paragraph.

Written Description – new matter

The rejection of claim 193 under 35 U.S.C. §112, first paragraph, written description requirement, is maintained for allegedly containing subject matter not described in the specification in such as way as to reasonably convey to one skilled in the relevant art that the inventors, at the

time the application was filed, had possession of the claimed invention. The Office alleges that claim 193 introduces new matter because it uses the phrase "polymer comprises nanoscale delivery vehicle."

The instant amendment addresses this issue. Express support in the disclosure for claims directed to methods for making a polypeptide polymer made by self-assembly of monomers, wherein the polypeptide polymer is a nanoscale drug delivery vehicle can be found, inter alia, on page 3, lines 17 to 18; page 89, lines 17 to 20; page 90, lines 17 to 20.

In light of the above remarks and the present claim amendments, Applicants respectfully submit that amended claims are fully enabled by and described in the specification to overcome the rejection based upon 35 U.S.C. §112, first paragraph.

CONCLUSION

In view of the foregoing amendment and remarks, Applicants respectfully aver that the Examiner can properly withdraw the rejection of the pending claims under 35 U.S.C. §112, first and second paragraphs. Applicants respectfully submit that all claims pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

Applicants believe that no additional fees are necessitated by the present response and amendment. However, in the event any such fees are due, the Commissioner is hereby authorized to charge any such fees to Deposit Account No. 13-1952 referencing docket no. 564462010900. Please credit any overpayment to this account.

As noted above, Applicants have requested a telephone conference with the undersigned representative to expedite prosecution of this application. After the Examiner has reviewed the instant response and amendment, please telephone the undersigned at (858) 720-5133.

Dated: April 6, 2005

Respectfully submitted,

By _____

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Short, et al.
Serial No.: 09/997,807
Filed: November 30, 2001

Art Unit : 1631
Examiner : Michael Borin, Ph.D.

Title: METHOD OF MAKING A PROTEIN POLYMER AND USES OF THE POLYMER

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Nelson Barton, am a co-inventor with Jay Short, Eric Mathur, W. Michael Lafferty and Kevin Chow, on the above-identified patent application.

2. I am an expert in the field of polymer chemistry and was an expert at the time of the invention. I am presently employed as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume as documentation of my credentials was attached to a Rule 132 expert declaration submitted in an earlier response.

3. Figure 1 in this application is an illustration of a transmission electron micrograph of a self-assembled protein polymer of the invention, where the protein polymer is comprised of a monomer having a sequence as set forth in SEQ ID NO:2, also called CanA.

4. At the time of the invention the skilled artisan, after reading the specification in its entirety, would have understand that CanA can mean either the exemplary polypeptide having a sequence as set forth in SEQ ID NO:2 (see e.g., page 17, line 58, and page 18; page 151, lines 14 to 16; page 152, lines 4 to 5, of the specification), or, an exemplary nucleic acid, such as SEQ ID NO:1, that encodes it (see, e.g., page 16, lines 12 to 48) - depending, of course, on the context in which the term CanA is used. Examples 19 and 20 of the specification both set forth experimentation demonstrating that SEQ ID NO:2, also called CanA, can self-assemble into polymers. These examples together with the specification, would have been sufficient to

demonstrate to one of skill in the art how to make and use the invention including the polymerization of CanA, or SEQ ID NO:2.

5. Figure 3A, 3B and 3C illustrates the process for preparing Example 21. As described in Example 21, polymerization of CanA monomer is initiated after formation of the liposome. This polymerization results in the extreme deformation of the liposomes, as illustrated in Figure 3B and 3C. Figures 3B and 3C illustrate a "liposome deformation" effect, where the liposome after polymerization of the polypeptide is deformed into a tubule-like shape, versus its natural un-deformed spherical shape. Without polymerization the lipids would remain spherical.

6. As evidence that the methods and materials set forth in the application as filed enable one of skill in the art to practice the invention as presently claimed, I have included (as Exhibit A) an immunofluorescent light microscope image of nanotubules assembled from a polypeptide conjugate of the present invention generated by attaching the CanA open reading frame (SEQ ID NO:1) to the open reading frame of the green fluorescent protein (GFP) ZSGREEN™ (BD Biosciences Clontech, Palo Alto, Calif.). These nanotubules are comprised of recombinant monomeric polypeptide subunit conjugates of SEQ ID NO:2 (CanA) attached to the green fluorescent protein. Self polymerization of the monomeric polypeptide subunit conjugates (the CanA- GFP chimeric protein) was achieved under the conditions set forth in Example 20.

7. It is my understanding that the Office has concerns as to whether Applicants have provided sufficient evidence that the level of skill in the art for self-assembly of conjugates with other biomolecules was high enough to enable self-assembly of any peptide conjugates without undue experimentation. The Office emphasized that the issue is not producing any peptide polymers, but rather whether there is an enabling disclosure for conditions for self-assembly. In my last declaration I declared that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for producing polypeptide polymers was very high. I would also like to emphasize that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for determining conditions for self-assembly of protein conjugates of the invention was also very high. It would have been routine for the skilled artisan at the time of the invention to screen for and select conditions that

facilitated self polymerization of the claimed conjugates of the invention, whether these composition comprised monomeric polypeptides having a modification comprising attachment of an enzyme, attachment of a nucleotide or attachment of a nucleotide derivative, or attachment of a lipid or attachment of a lipid derivative, or attachment of a targeting molecule, or attachment of a vector.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 3/24/05

Nelson Barton

Figure 3. Immunofluorescent light microscope image of nanotubules assembled from a fusion protein created by fusing the CanA open reading frame to the open reading frame of the green fluorescent protein ZsGreen (BD Biosciences Clontech, Palo Alto, CA).